

THE QUALITATIVE ANALYSIS OF ORGANIC SUBSTANCES

INTRODUCTION

A FUNDAMENTAL difference exists between the qualitative analysis of inorganic materials and the identification of organic substances. Inorganic compounds are, for the most part, ionic, and since the number of different ionic species which may be encountered is relatively limited, it is possible to develop a set scheme, by adherence to which a complete analysis may be achieved. Organic compounds, on the other hand, are essentially covalent, and in consequence each one of the enormous number of known compounds is a separate entity, and no rigid scheme can be developed which is applicable to all cases. The method outlined here should therefore be taken only as a guide, since substances may be encountered which show exceptional behaviour. The student requires to have a sound knowledge of the properties of organic compounds, and must use this knowledge to the fullest extent in order to arrive at the correct interpretation of the experimental results.

SUGGESTED PROCEDURE

The method of investigation may be divided roughly into four parts:

- (1) Preliminary investigation of the physical properties, and the physical separation of mixtures where applicable.
- (2) Detection of the elements present.
- (3) Detection of characteristic groupings, and the chemical separation of mixtures.
- (4) Preparation of derivatives for complete characterization.

PRELIMINARY INVESTIGATION

An examination of the physical properties will often indicate whether the material is a single substance or a mixture. In the

2

Preliminary Investigation

case of a solid, a small specimen is heated on a spatula; if it is seen to melt, a melting-point determination is carried out in the usual way. A sharp melting-point usually indicates a single substance. A specimen of the material should be recrystallized, a suitable solvent being found by trial. If the melting-point of the recrystallized material is not more than $3-4^{\circ}$ higher than the original melting-point, it is unlikely that a mixture is present. If a large change in melting-point is observed, a mixture is probable, although such a change could also be caused by solvation of crystals or by reaction between the substance and the solvent; for example, recrystallization of an acid anhydride from water will yield the acid.

In the case of a liquid, a specimen should be distilled. If decomposition occurs at ordinary pressure (preliminary test), the pressure should be reduced. If the whole, or almost the whole, of the substance distils at a sensibly constant temperature (not more than 5° range), then it may be a single substance, although a mixture is not excluded. Distillation over a considerable range of temperature indicates a mixture; in many cases it may be possible to separate the material into two clearly defined fractions. In such a case, the whole sample should be carefully fractionated, and each fraction redistilled; the use of a short column is advantageous, provided that the boiling-point is not very high.

A liquid mixture which cannot be separated readily by fractionation may be separable by chemical means. Such separations involve a knowledge of the functional groups present and must therefore be deferred to a later stage in the investigation (see p. 18). Solid mixtures, also, are usually most satisfactorily separated by chemical means; if no such method can be found, recourse must be made to physical methods, such as utilization of differences of solubility of the components in different solvents, or the sublimation or steam distillation of one component.

If the equipment is available, preparative vapour-phase chromatography may provide an excellent means of separation, particularly for liquid mixtures. Adsorption and partition chromatography can also be applied, but are time-consuming. Paper or thin-layer chromatography may be useful in following the course of a separation. It should be remembered, however, that these sensitive methods will show the presence of impurities in most commercial chemicals.

Detection of Elements

3

DETECTION OF CONSTITUENT ELEMENTS

It is not usual to test for **carbon** and **hydrogen** in a substance which is known to be organic.

A little of the substance should be ignited on a crucible lid until all the carbon has burnt away. In difficult cases, cooling, moistening the black residue with ammonium nitrate solution and re-igniting may assist. If a white or coloured residue remains, the presence of a **metal** is indicated. Its nature should be determined by applying the methods of inorganic analysis to the residue.

The presence of **nitrogen**, **sulphur** and the **halogens** is detected by the **Lassaigntest**. A small piece of clean sodium, about the size of a rice-grain, is placed in a $3 \times \frac{3}{8}$ in. test-tube, which is held in a pair of tongs. About 2 drops of the substance, if a liquid, or 0.1 g. if a solid, is added, and the tube is gently warmed. Any vigorous reaction is allowed to subside, and the tube is then heated more and more strongly, until finally a red heat is attained. After maintaining this temperature for 1–2 min., the tube is plunged into 15 c.c. of distilled water contained in a small beaker; the tube crumbles and the remaining sodium dissolves with a flash. This process should always be carried out in the fume cupboard, with the front drawn down to protect the operator. If the substance under investigation refuses to react under these conditions, the procedure may be slightly modified. The substance is introduced into the tube, and mixed with an excess (about 0.3 g.) of pure, anhydrous sodium carbonate. The mixture is covered with a layer of sodium carbonate, and the piece of sodium is placed on top of this. The tube is now heated at the level of the sodium, until the latter melts and begins to vaporize. The heating is gradually extended downwards, so that the substance is slowly volatilized over the hot sodium. Finally, the whole mass is brought to red heat. The further procedure is the same as before.

The fusion converts any nitrogen to sodium cyanide, sulphur to sodium sulphide and halogens to sodium halides; these dissolve when the tube is plunged into water. After stirring and gentle warming, the aqueous solution is filtered. It should be quite colourless; if it is coloured, the heating with sodium was not strong

4

Detection of Elements

enough and the test must be repeated. A coloured solution cannot be relied on to give the correct result. The solution is divided into portions and tested as follows:

Sulphur. To a portion of the alkaline solution, 1 drop of a freshly prepared aqueous solution of sodium nitroprusside is added. If any sulphur is present, a deep red-violet colour will be produced. Alternatively, the presence of sulphide ion may be determined by adding lead acetate solution to a portion of the solution which has been acidified with acetic acid.

Nitrogen. To a portion of the solution are added a few drops of a freshly prepared solution of ferrous sulphate; a dark green precipitate of ferrous hydroxide is formed. (If sulphur is present, the precipitate will be black; rather more ferrous solution should be added in this case.) The mixture is heated to boiling, with shaking, cooled and acidified with dilute sulphuric acid. If nitrogen is present, a blue or green colour (Prussian blue) appears immediately, or on the addition of a *trace* of ferric chloride solution. If any doubt exists about the presence of Prussian blue the solution should be filtered. The Prussian blue precipitate, if present, is then retained by the filter and is clearly visible against the white background. In the absence of nitrogen, a pale yellow solution is obtained.

Halogens. The remainder of the solution is acidified with dilute sulphuric acid, and, if nitrogen or sulphur has been found, it is boiled for 2 min. to expel HCN and H₂S. Silver nitrate solution is then added to a portion; a precipitate indicates halogen. A blank test should be carried out on the reagents used. To distinguish the individual halogens, a drop of carbon tetrachloride is added to a fresh portion of the solution, and *very dilute* sodium hypochlorite is added slowly with shaking. Iodine gives a violet colour in the carbon tetrachloride, discharged by excess hypochlorite; bromine gives a yellow colour, stable to excess reagent. Chlorine is detected in the presence of bromine or iodine by boiling a portion of the original solution with an equal volume of concentrated nitric acid for 5 min., then adding silver nitrate.

Beilstein's test is useful as a confirmation. A copper wire is heated in a Bunsen flame until it no longer imparts a green

Detection of Characteristic Groupings 5

coloration to the flame. It is then allowed to cool, and a little of the substance is placed on it. On again heating in the flame, if halogen is present a transient green or blue coloration will be produced. As this test is very sensitive, and is also given by some compounds containing no halogen, it should be used only to confirm the *absence* of halogen; contact of the heated wire with anything except the substance under test must be avoided.

Other elements. It is not usual to test for other elements (e.g. P, As) unless there is some reason to suspect their presence.

The most general procedure consists in heating a small quantity of the material with a considerable excess of concentrated sulphuric acid until strong fuming occurs. If the solution remains coloured, it is cooled somewhat, and a few drops of concentrated nitric acid are carefully added. Heating is then continued until the mixture is colourless. The cooled solution is poured into water, and tested for the presence of phosphate, arsenate, or other inorganic ions.

DETECTION OF CHARACTERISTIC GROUPINGS

A variety of reactions may be used for determining to what class of compound an organic material belongs. Those given here have been chosen for simplicity of application and reasonable reliability.

The tests should be applied in the order given. In the case of mixtures, once a functional group has been detected, use should be made of it to separate the components of the mixture (see p. 18), before the further tests are carried out. After the separation, the Lassaigne test should be applied to each pure component, unless a completely negative test was obtained in the first place. Once a functional group has been detected, regard must be taken of its possible influence on the applicability of subsequent tests. In the following sections, the further tests which may be usefully applied are listed at the end of each subsection. When the general nature of the substance has been ascertained, confirmatory tests should be applied whenever possible.

Infra-red and NMR spectroscopy are valuable methods for detecting or confirming the presence of certain functional groups and structural features.

6 *Compounds containing C, H and O only*

SECTION A. COMPOUNDS CONTAINING CARBON, HYDROGEN AND OXYGEN ONLY

(i) **Observe the solubility of the substance in water and in ether**

Solubility is, of course, a relative term, but there is little to be gained by specifying a particular figure above which a substance is said to be 'soluble'. The student should note the approximate degree of solubility of the material in the two solvents, and his conclusions must be governed by his observations. Care must be taken not to confuse a slow rate of solution with insolubility; the material, if solid, should be powdered as finely as possible. If the substance is insoluble in cold water, test its solubility in hot water.

In dealing with a mixture, it is not always easy to detect by simple observation whether or not one of the components is soluble. Hence, with a liquid mixture, a definite volume should be taken, and any diminution in this volume carefully observed. With a solid mixture, filtration, followed by evaporation of the filtrate, will serve to indicate whether any of the material has dissolved; a change in melting-point of the undissolved portion (after thorough drying) may also indicate that some of the material has dissolved.

Substances which are very **soluble in both solvents** are normally of low molecular weight, and are of a fairly polar nature; e.g. the lower aliphatic alcohols, amines, aldehydes and ketones, acids, nitriles and esters, and also a few phenols. The aqueous solution should be tested with litmus paper.

Substances which are **soluble in water and insoluble in ether** are of a highly polar character. They include salts of acids and bases, polyhydroxy compounds (e.g. carbohydrates), sulphonic acids, some hydroxy-acids and poly-carboxylic acids, aliphatic amino-acids, etc. To compounds of this type containing C, H and O only, apply tests (ii) and (iiib), and if negative results are obtained, test for carbohydrates (see p. 53).

A very large number of substances are **insoluble in cold water and soluble in ether**. These compounds are either non-polar (hydrocarbons, halides), or have a high proportion of 'non-polar' to 'polar' groupings (higher aliphatic compounds, most aromatic compounds). Some of the latter type are soluble in hot water.

Compounds containing C, H and O only 7

Those compounds, which are **insoluble in both solvents**, are mainly of a weakly polar or non-polar nature and high molecular weight, or are water-insoluble salts.

(ii) Observe whether the substance liberates carbon dioxide from saturated aqueous potassium bicarbonate solution

If the substance is very insoluble in water, an alcoholic solution may be used (this will cause some precipitation of potassium bicarbonate). The mixture must not be heated.

A positive test is given by **carboxylic acids** and by most **acid chlorides** and **anhydrides**. Sulphonic acids and most nitrophenols also react. In alcoholic solution some polyhalogenated phenols react. Salts of weak bases give a positive test, and if the base is insoluble it is precipitated. Exceptions: aliphatic aminoacids and some long-chain fatty acids do not react.

All substances which give a positive test can be titrated against caustic soda solution, using phenolphthalein as indicator, and the neutralization equivalent thus determined.

If positive, apply tests (iii*b*), (v), (vi) and (xi).

(iii*a*) If the substance is insoluble, or only sparingly soluble, in water, test its solubility in dilute (5%) aqueous caustic soda. Filter, if necessary, and reacidify

This test detects weakly acidic substances: **phenols**, **enols**, and **long-chain fatty acids** (soapy solution). A few **readily hydrolysed esters** (e.g. ethyl oxalate) also dissolve, but they are not reprecipitated on acidification. **Acid anhydrides** dissolve slowly; on acidification the acid will be obtained. Some phenols give sparingly soluble sodium salts, but dissolve sufficiently to form a precipitate on re-acidification.

If positive, apply tests (v), (vi) and (xi).

(iii*b*) If the substance is readily soluble in water (or if one component is soluble) prepare an approximately 1% aqueous solution, and add 1 drop of ferric chloride solution

The production of a colour (other than yellow), which may be transient, indicates the presence of a phenolic or enolic substance.

8 *Compounds containing C, H and O only*

α -Hydroxy-acids give a strong yellow colour. All phenols and enols which are too soluble in water for test (iii a) to be applicable give intense ferric reactions, with the exception of hydroquinones, which are oxidized to the quinone (characteristic smell). Enols are distinguished from phenols by test (v). Amines may precipitate ferric hydroxide.

(iv) If the material is insoluble in water, test its solubility in dilute hydrochloric acid. Filter, if necessary, and make the filtrate alkaline

This test detects the presence of water-insoluble bases. These are almost invariably nitrogenous bases. Even if nitrogen has not been detected, however, this test should be performed, since some amines (especially tertiary amines) react with difficulty in the Lassaigne test. Many amines of higher molecular weight yield rather insoluble hydrochlorides and hence may appear to be insoluble in the acid, but usually sufficient of the salt remains in solution to produce a turbidity or precipitate when the filtered solution is made alkaline. Dilute sulphuric acid should not be used for this test, as the amine sulphates are in most cases less soluble than the hydrochlorides.

Water-soluble amines obviously cannot be detected by this procedure, but are usually easily detected by their typical odour, and by the alkaline reaction of their aqueous solutions.

Diaryl- and triaryl-amines, and some negatively substituted aromatic amines, are too weakly basic to dissolve in dilute hydrochloric acid. Salts of carboxylic acids liberate the parent acid.

If positive, re-test for nitrogen and see p. 12.

(v) Test for the presence of carbonyl groups by treating 2 drops or 0.1 g. of the substance, dissolved in the minimum of methanol, with 3 c.c. of Brady's reagent (see p. 46). If no immediate reaction occurs, boil for 2 min., cool, and scratch with a glass rod.

Almost all carbonyl compounds (and most acetals) react readily with dinitrophenylhydrazine sulphate, giving yellow to red precipitates. If a positive result is obtained, apply tests (vi) and (xi), and also the following supplementary tests:

Compounds containing C, H and O only 9

(a) Dissolve the material in ether, and shake with a saturated solution of sodium bisulphite. Aldehydes, most alkyl methyl ketones (but not aryl methyl ketones), cyclic ketones and α -diketones readily yield crystalline bisulphite compounds. Benzo- and naphtho-quinones dissolve in aqueous sodium bisulphite, being converted into water-soluble quinolsulphonic acids.

(b) *Tollens's test.* Place 2 c.c. of 5% aqueous silver nitrate in a thoroughly clean test-tube, and add 1 drop of dilute caustic soda solution. Add dilute (*ca.* 2%) ammonia dropwise until the precipitate just redissolves; excess of ammonia must be avoided. Add 1 or 2 drops (or 0.1 g.) of the substance and shake. If no reaction occurs in the cold, warm gently, but do not boil. Aldehydes produce a silver mirror; ketones (except α -diketones and α -hydroxyketones) do not react. Other reducing substances, such as hydrazines and hydroxylamines, amino-phenols, etc., also produce mirrors.

The Tollens reagent must not be kept, as it slowly forms very explosive substances. It must be freshly prepared for use, and washed away after the reaction has been carried out.

(vi) Test for the presence of ester linkages

To 1 drop of the material add 3 drops of saturated alcoholic hydroxylamine hydrochloride and 3 drops of 20% methanolic potash, heat to boiling, cool, acidify with $N/2$ hydrochloric acid, and add ferric chloride solution dropwise. A deep red or purple colour indicates the presence of an ester. Although 1 drop of ferric chloride is usually sufficient, oxalic esters may require the addition of several drops of the reagent. The test depends on the formation of a hydroxamic acid, $R.C(:NOH).OH$, which gives a coloured ferric salt. **Acid halides** and **anhydrides** also give a positive test.

A positive result should be confirmed by conducting a quantitative saponification (see p. 59). Also apply test (xi).

(vii) Test for alcoholic hydroxyl groups

It is essential that the material should be quite free from water before applying the following tests. Liquids should be dried with freshly calcined potassium carbonate for as long as possible, with frequent shaking.

10 *Compounds containing C, H and O only*

(a) *Acetyl chloride test* (applicable only if tests (ii), (iii) and (iv) are negative). To a few drops of the substance add half the volume of acetyl chloride. Shake the mixture gently, and observe whether any heat is developed, and whether considerable evolution of hydrogen chloride occurs. In the case of a solid substance, if no obvious reaction occurs, reflux the mixture for a few minutes, pour into water, and compare the melting-point of the recovered with that of the initial material. Tertiary alcohols evolve no hydrogen chloride, since they are converted to the chlorides by acetyl chloride.

(b) *Sodium test* (applicable only if tests (ii)–(vi) are negative). To about 1 c.c. of the liquid, add a small piece of sodium, freshly cut under toluene. If no immediate reaction occurs, warm the liquid to about 80°. If the material is alcoholic, the sodium should react steadily with the evolution of hydrogen, and if a sufficiently small piece was used, it should eventually dissolve completely. Frequently, the sodium alkoxide separates as a colourless solid. If the alcohol has a very high boiling-point, the reaction may be very slow. Any slight effervescence which occurs immediately the sodium is added, but which quickly slackens, should be disregarded; it is due to a trace of moisture introduced with the sodium. Solids having a melting-point greater than 50° can be tested by first dissolving them in the minimum of dry benzene; in such cases, however, reaction with sodium will usually be extremely slow, and the test is not reliable under these conditions. Sodium also reacts with mono-substituted acetylenes.

If test is positive, apply test (xi).

(viii) **Test for acetal groupings**

Acetals are hydrolysed readily by acids, but not by alkalis. A specimen of the material should be heated with dilute sulphuric acid. The formation of a volatile aldehyde is usually easily detected by the smell; the vapours should be collected by absorption in a little methanol and tested with Brady's reagent. Any residual oil should be tested for the presence of a non-volatile aldehyde. Most acetals react directly with Brady's reagent on heating.